(57%) of pale yellow crystals of V, mp 234–236 °C dec, which was identical with that obtained by method A.

Method C. A solution of 197 mg (0.72 mmol) of carbodithioate II and 170 mg (0.72 mmol) of thiosemicarbazide VI in 30 mL of MeCN was heated at reflux for 8 h. The reaction mixture was chilled, and the crystals that separated were collected to give 188 mg (62%) of pale yellow crystals of V, mp 235–237 °C dec, which was identical with that obtained by method A.

1-(2-Pyridinyl)piperazine-4-thiocarboxylic Acid Hydrazide (VI). A solution of 181 mg (1 mmol) of 4-methyl-4-phenyl-3-thiosemicarbazide[13] and 163 mg (1 mmol) of 1-(2-pyridinyl)piperazine in 5 mL of MeOH was heated at reflux for 3 h. The solution was chilled, and the crystals that separated were collected to give 115 mg (48%) of colorless needles of VI: mp 184–185 °C (from MeOH); IR 3270, 3220, 3010, 2880, 1600, 1550, 1488, 1445, 1231, 1210, 982, 946, 768, 728 cm^{-1}. Anal. ($C_{10}H_{15}N_5S$) C, H, N, S.

Methyl 3-[1-(1-Isoquinolinyl)ethyl]hydrazinecarbodithioate (VIII). A suspension of 10 g (36.3 mmol) of carbodithioate II in 100 mL of EtOH was treated portionwise with 2.5 g (66 mmol) of $NaBH_4$, and the mixture was stirred for 2 h. An additional 2.5 g (66 mmol) of $NaBH_4$ was added, and stirring was continued for 2 h. The solution was poured into 100 mL of H_2O and treated cautiously with 6 mL of glacial HOAc. The gum that separated was extracted into 100 mL of $CHCl_3$, the extract was washed with three 100-mL portions of H_2O, and the dried $CHCl_3$ solution was evaporated under reduced pressure. The residual oil was rubbed under 25 mL of cold EtOH, and the crystals that formed were collected. This afforded 7.5 g (75%) of colorless rosettes, mp 107–109°C. An analytical sample of VIII was prepared by crystallization from MeCN: mp 109–110 °C; IR 3230 (NH), 3140, 2978, 1622, 1590, 1535, 1449, 1305, 1101, 1045, 1005, 818, 743 cm^{-1}. Anal. ($C_{13}H_{15}N_3S_2$) C, H, N, S.

3-Azabicyclo[3.2.2]nonane-3-thiocarboxylic Acid 2-[1-(1-Isoquinolinyl)ethyl]hydrazide (16). The preparation of 1-[1-(1-isoquinolinyl)ethyl]thiosemicarbazides from carbodithioate VIII is exemplified by the following reaction. A solution of 4.85 g (17.3 mmol) of VIII in 15 mL of EtOH was treated with 2.35 g (18.8 mmol) of 3-azabicyclo[3.2.2]nonane, and the mixture was heated under reflux for 8 h. The solution was chilled, and the product that separated was collected, affording 4.3 g (72%) of colorless cubes of **16**. An analytical sample was prepared by two crystallizations from MeCN: IR 3220 (NH), 3170 (NH), 2930, 1619, 1583, 1559, 1480, 1345, 1282, 825, 749 cm^{-1}. Anal. ($C_{20}H_{26}N_4S$) C, H, N, S.

Biological Method. The compounds described herein were tested at the Leo Rane Laboratory, University of Miami, Miami, FL, against a drug-sensitive strain of *Plasmodium berghei* (strain KBG 173) in mice. Details of the test procedure are given in the first paper in this series[4] and by Osdene, Russell, and Rane.[14]

Registry No. **1**, 87555-46-2; **2**, 87555-47-3; **3**, 87555-48-4; **4**, 85748-57-8; **5**, 75013-89-7; **6**, 87555-49-5; **7**, 87555-50-8; **8**, 87555-51-9; **9**, 87555-52-0; **10**, 87555-53-1; **11**, 87555-54-2; **12**, 87555-55-3; **13**, 87555-56-4; **14**, 87555-57-5; **15**, 87555-58-6; **16**, 87555-59-7; **17**, 87555-60-0; **18**, 87555-61-1; **19**, 87555-62-2; II, 85748-38-5; IV, 87555-63-3; V, 87555-64-4; VI, 87555-65-5; VIII, 87555-66-6; 1-acetylisoquinoline, 58022-21-2; 4-methyl-4-phenyl-3-thiosemicarbazide, 21076-05-1; methyl hydrazinecarbodithioate, 5397-03-5.

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2-Acetylpyridine Thiosemicarbazones. 9. Derivatives of 2-Acetylpyridine 1-Oxide as Potential Antimalarial Agents[1,2]

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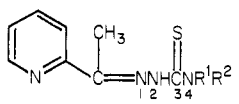
In view of the antimalarial activity in mice of 2-acetylpyridine thiosemicarbazones, a series of analogous 1-oxides was prepared for evaluation. Their synthesis was achieved by the reaction of 2-acetylpyridine 1-oxide with methyl hydrazinecarbodithioate to give methyl 3-[1-(2-pyridinyl 1-oxide)ethylidene]hydrazinecarbodithioate (II). Reaction of the latter intermediate with secondary amines afforded the desired 2-acetylpyridine 1-oxide thiosemicarbazones (III). Reduction of the azomethine linkage of II with $NaBH_4$ gave methyl 3-[1-(2-pyridinyl 1-oxide)ethyl]-hydrazinecarbodithioate (IV) whose *S*-methyl group was then displaced by amines to give a 1-[1-(2-pyridinyl 1-oxide)ethyl]thiosemicarbazide, V. Antimalarial activity of III was evaluated against both *Plasmodium berghei* in the mouse and *Plasmodium falciparum* in an automated in vitro test system. In both cases, 2-acetylpyridine 1-oxide thiosemicarbazones were found to be less active than the corresponding de-1-oxide analogues. When compounds V were evaluated against *Plasmodium berghei* in the mouse, a diminution of activity was similarly seen in comparison to the analogues not bearing the 1-oxide moiety.

Various 2-acetylpyridine thiosemicarbazones (I) possess



I

antitrypanosomal,[3] antibacterial,[4-7] antiviral,[8] and antileukemic[9,10] properties. Inasmuch as our attention has centered upon the antimalarial effects of these compounds,[11,12] their analogues,[2,13-16] and their derivatives,[9] a program of molecular modification has been undertaken

(1) This is contribution no. 1684 to the Army Research Program on Drug Development.

(2) For paper no. 8 in this series, see: Klayman, D. L.; Scovill, J. P.; Bruce, J.; Bartosevich, J. F. *J. Med. Chem.*, preceding paper in this issue.

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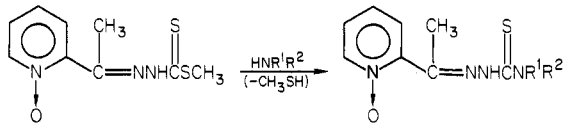
in order to develop agents with greater efficacy against drug-resistant malaria. Certain molecular features have been shown to be essential for antimalarial activity in this class of compounds,[2,16] and since these thiosemicarbazones are excellent chelating agents, it appears that those structural alterations that abolish their affinity for transition metals also tend to destroy their biological activity.

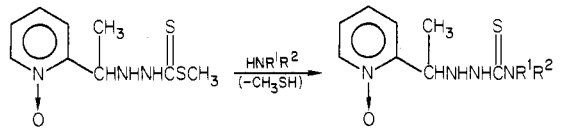
Pyridine 1-oxide has been reported to be an effective ligand for transition-metal ions.[17] We felt, therefore, that thiosemicarbazones derived from 2-acetylpyridine 1-oxide were likely to retain the high affinity for transition-metal ions displayed by the parent 2-acetylpyridine thiosemicarbazones and, thus, their antimalarial properties. In this paper we describe the synthesis and evaluation of the 1-oxides and, inasmuch as thiosemicarbazides derived by the reduction of antimalarial thiosemicarbazones were observed previously to retain their activity as antimalarials,[16] the synthesis and evaluation of 1-[1-(2-pyridinyl 1-oxide)ethyl]thiosemicarbazides. Finally, the copper complex of a 2-acetylpyridine 1-oxide thiosemicarbazone was prepared to compare its activity with the identical copper complex lacking the 1-oxide moiety.

Chemistry. The reaction of 2-acetylpyridine 1-oxide with methyl hydrazinecarbodithioate afforded methyl 3-[1-(2-pyridinyl 1-oxide)ethylidene]hydrazinecarbodithioate (II). The *S*-methyl group of this dithio ester is



II III

readily displaced by primary and secondary amines, yielding the desired 2-acetylpyridine 1-oxide thiosemicarbazones, III (cf. Table I). Reduction of the azomethine linkage of II with $NaBH_4$ gave methyl 3-[1-(2-pyridinyl 1-oxide)ethyl]hydrazinecarbodithioate (IV). There was



IV V

no evidence for formation of any de-1-oxide product. Reaction of dithio ester IV with an amine afforded 1-[1-(2-pyridinyl 1-oxide)ethyl]thiosemicarbazides, V (cf. Table II).

Katritzky and Hand[18] have reported that in the IR the N–O stretching band of 2-substituted pyridine 1-oxides appears between 1274 and ~1220 cm^{-1} ($CHCl_3$). This region of the spectra of 2-acetylpyridine thiosemicarbazones is already occupied by an intense band(s) and, therefore, could not be used for diagnostic purposes of their 1-oxide derivatives (III). However, the related thiosemicarbazides do not show strong absorption in this area, and, indeed, their 1-oxide derivatives (V) show a sharp peak (the most intense in the spectrum) in the region 1236–1226 cm^{-1} (KBr).

The 2-acetylpyridine 1-oxide thiosemicarbazone, 3-azabicyclo[3.2.2]nonane-3-thiocarboxylic acid 2-[1-(2-pyridinyl 1-oxide)ethylidene]hydrazide (14), was combined with $CuCl_2$ to give a bronze-colored product, **15**, which was shown by microanalysis to correspond to a 1:1:1 complex of ligand, metal ion, and gegenion.

Results and Discussion

Antimalarial activity of the 2-acetylpyridine 1-oxide thiosemicarbazones was evaluated in two test systems, namely, an in vivo mortality-based assay against *Plasmodium berghei* in the mouse and an automated in vitro test against *Plasmodium falciparum*. In the latter test system, two strains were utilized, the multidrug-resistant Vietnam Smith strain and the chloroquine-susceptible, pyrimethamine-resistant Camp strain.

The antimalarial properties of 2-acetylpyridine thiosemicarbazones against *Plasmodium berghei* in the mouse have been previously reported by us.[16,19] The most active compound in this series was 1-(2-pyridinyl)piperazine-4-thiocarboxylic acid 2-[1-(2-pyridinyl)ethylidene]hydrazide, which cured three out of five mice at a dose of 20 mg/kg and five out of five mice at 80 mg/kg. Toxicity was observed at 320 mg/kg, with deaths occurring in two out of five mice. The corresponding 1-oxide derivative (11) shows no evidence of toxicity at 640 mg/kg and cured two out of five mice at this dose level. The most active of the 2-acetylpyridine 1-oxide thiosemicarbazones is compound 10, a derivative of 1-(ethoxycarbonyl)piperazine. This compound cured one out of five animals of 80 mg/kg and three out of five mice at 160 mg/kg and is toxic to five out of five animals at 320 mg/kg. Only compound **5** proved capable of producing a 100% cure response, this at the level of 640 mg/kg. The corresponding 2-acetylpyridine-thiosemicarbazone cured three out of five mice at 40 mg/kg and caused toxic deaths to five out of five mice at 160 mg/kg.

We have recently reported on the antimalarial activity of 1-[1-(2-pyridinyl)ethyl]thiosemicarbazides, which may be regarded as being derived from the corresponding thiosemicarbazones by reduction of the azomethine moiety. These compounds were seen to be somewhat more active than the thiosemicarbazones from which they were derived; however, this increase in activity is accompanied by a substantial increase in toxicity.[16] The most active member of this class of compounds was 3-azabicyclo[3.2.2]nonane-3-thiocarboxylic acid 2-[1-(2-pyridinyl)ethyl]-hydrazide, which cured two out of five animals at 10 mg/kg and four out of five mice at 20 mg/kg and produced five toxic deaths in five mice at 40 mg/kg. The 1-oxide analogue (cf. Table III), compound **21**, cured one out of five animals at 640 mg/kg. This compound was the only one

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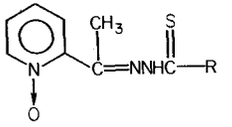
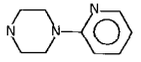
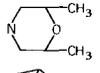
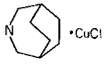
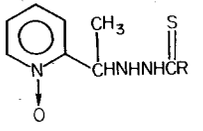
Table I. Antimalarial Activity of 2-Acetylpyridine 1-Oxide Thiosemicarbazones against *Plasmodium berghei* in the Mouse

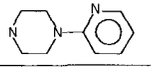


no.	R	mp, °C	formula	recrystn solvent	yield, %	increase in mean survival time, days, and no. of cures at the following dosage, mg/kg[a] 40	80	160	320	640
1	$N(CH_3)_2$	161–162	$C_{10}H_{14}N_4OS$	EtOH	90	2.3	0.7	4.2, T(3/5)	T(5/5)	6.9A, T(4/5)
2	$N(CH_2)_4$	207–208	$C_{12}H_{16}N_4OS$	EtOH	91	0.9	1.1	1.5	C(2/5)	5.9
3	$N(CH_2)_5$	193–194	$C_{13}H_{18}N_4OS$	EtOH	90	3.5	1.3	6.3A	C(1/5)	C(3/5)
4	$N(CH_2)_6$	195–197	$C_{14}H_{20}N_4OS$	EtOH	92	0.9	0.7	3.1	4.9	7.1A
5	c-NC_5H_9-2-CH_3	180–182	$C_{14}H_{20}N_4OS$	EtOH	89	2.3	3.3	C(1/5)	C(2/5)	C(5/5)
6	c-NC_5H_9-3-CH_3	203–204	$C_{14}H_{20}N_4OS$	EtOH	94	1.5	1.6	4.7	1.4	C(1/5)
7	c-NC_5H_9-4-CH_3	191–192	$C_{14}H_{20}N_4OS$	EtOH	83	0.6	2.2	2.3	4.2	C(1/5)
8	c-$N(CH_2CH_2)_2S$	212–214	$C_{12}H_{16}N_4OS$	DMF	76	0.1		0.9		1.5
9	c-$(CH_2CH_2)_2N$-CHO	213–214	$C_{13}H_{17}N_5O_2S$	DMF	20	−0.3		0.3		T(2/5)
10	c-$N(CH_2CH_2)_2N$-$CO_2C_2H_5$	213–214	$C_{15}H_{21}N_5O_3S$	EtOH	87	4.7	C(1/5)	C(3/5)	T(5/5)	T(5/5)
11		200–203	$C_{17}H_{20}N_6OS$	EtOH	90	0.5	0.3	1.9	C(4/5)	C(2/5)
12	c-$N(CH_2CH_2)_2N$-C_6H_5	212–213	$C_{18}H_{21}N_5OS$	EtOH	90	0.1	1.7	3.5	9.1A	8.3A
13		207	$C_{14}H_{20}N_4O_2S$	EtOH	70	4.9	6.1	6.1		C(1/5)
14		200	$C_{16}H_{22}N_4OS$	EtOH	91	−0.7	0.1	1.1	4.9	C(2/5)
15		238–239 dec	$C_{16}H_{21}ClCuN_4OS$	DMF	88	−0.3		1.4, T(1/5)		2.4, T(2/5)

[a] A = active; C = cure; T = toxic. These terms are defined in ref 11 and 22.

Table II. Antimalarial Activity of 1-[1-(2-Pyridinyl 1-oxide)ethyl]thiosemicarbazides against *Plasmodium berghei* in the Mouse



no.	R	mp, °C	formula	recrystn solvent	yield, %	increase in mean survival time, days, and no. of cures at dosage, mg/kg[a] 20	40	80	160	320	640
16	$N(CH_2)_4$	210	$C_{12}H_{18}N_4OS$	EtOH	66		0.8		0.0		1.6
17	$N(CH_2)_5$	198–200	$C_{13}H_{20}N_4OS$	EtOH	92		0.2		0.2		1.8
18	$N(CH_2)_6$	178–180	$C_{14}H_{22}N_4OS$	MeOH	58		−0.2	0.4	0.8	1.6, T(1/5)	3.5, T(2/5)
19		191–192	$C_{16}H_{24}N_4OS$	MeCN	81	−0.1	−0.3	0.7	0.9	2.5	C(1/5)
20	c-$N(CH_2CH_2)N$-$CO_2C_2H_5$	178	$C_{15}H_{23}N_5O_3S$	EtOH	65	0.4	1.2	6.0	6.6A	8.0A, T(2/5)	9.0A, T(4/5)
21		204–205	$C_{17}H_{22}N_6OS$	DMF	81		−0.2		0.2		3.4

[a] A = active; C = cure; T = toxic. These terms are defined in ref 11 and 22.

Table III. Antimalarial Activity of 2-Acetylpyridine 1-Oxide Thiosemicarbazones against *Plasmodium falciparum* in Vitro

no.	ID_{50}, ng/mL	
	Smith strain	Camp strain
1	78 (3.6)[a]	54
2	44 (4.1)	48
5	80 (13)	77 (15)
6	51 (17)	39
7	73 (12)	74
8	54	44
10	inactive (4.8)	inactive (5.4)
11	inactive (18)	inactive (10)
12	inactive (17)	62
13	46	41
14	83 (10)	86 (13)

[a] Values in parentheses are those obtained for the corresponding 2-acetylpyridine thiosemicarbazones (ref 19).

in its class that had curative properties. Two compounds, 18 and **20**, produced toxic deaths at doses of ≥320 mg/kg.

The antimalarial activity of the copper complex 15 was substantially lower than that of the free ligand, 14. No activity was observed at doses of up to 640 mg/kg, with two out of five toxic deaths occurring at this dose level. The corresponding de-1-oxide complex cured two out of five test animals at a dose of 80 mg/kg five out of five at 160 mg/kg and was toxic to four out of five mice at a dose of 320 mg/kg.[9]

We have previously reported on the in vitro antimalarial activity of 2-acetylpyridine thiosemicarbazones in which we observed that the test compounds were equally effective against the Smith and Camp strains.[19] Selected 2-acetylpyridine 1-oxide thiosemicarbazones were evaluated in the present study against these parasites (cf. Table III). 2-Acetylpyridine N^4,N^4-dimethylthiosemicarbazone proved to be the most active of the 2-acetylpyridine N^4,N^4-disubstituted thiosemicarbazones that we tested in this screen, having an ID_{50} of 3.6 ng/mL against the Smith strain of *P. falciparum*. The corresponding 2-acetylpyridine 1-oxide thiosemicarbazone (1) proved to be much less active, having an ID_{50} of 78 ng/mL against this parasite and an ID_{50} of 54 ng/mL against the Camp strain. For comparison, it is of interest to note that the clinically useful antimalarial agent chloroquine had an ID_{50} of 60 ng/mL against the Smith strain and an ID_{50} of 7.6 ng/mL against the Camp strain. The most active 2-acetylpyridine 1-oxide thiosemicarbazone was the 2,6-dimethylmorpholine derivative (13). This compound showed an ID_{50} of 46 ng/mL against the Smith strain and an ID_{50} of 41 ng/mL against the Camp strain. The in vitro antimalarial activity of the 1-oxide derivatives is lower by a factor of 3–10 than the corresponding 2-acetylpyridine thiosemicarbazones whenever a comparison is possible (cf. Table III). Both strains of *P. falciparum* seem to be equally susceptible to the inhibitory effects of the test compounds.

In general, the 2-acetylpyridine 1-oxide thiosemicarbazones are less potent antimalarials and less toxic than their parent compounds lacking the 1-oxide moiety.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus. Infrared spectra were recorded as KBr disks on a Perkin-Elmer Model 283 spectrophotometer. NMR spectra were run on either a JEOL FX90Q or Varian T-60A spectrometer in $CDCl_3$ with tetramethylsilane as internal standard. Microanalyses were performed by the Spang Microanalytical Laboratory, Eagle Harbor, MI. Satisfactory microanalyses (±0.3% of calculated values) were obtained for all compounds.

2-Acetylpyridine 1-Oxide. This compound was made by the oxidation of 2-acetylpyridine with 30% H_2O_2 in glacial HOAc using the method of Winterfeld and Zickel:[20] bp 104–106 °C (0.4 mmHg), rapidly became a semisolid at room temperature [lit. bp 96–97 °C (0.2 mmHg); mp 35–36 °C;[19] bp 82 °C (bath) (0.3 mmHg);[21] IR (neat) 3105, 1685, 1600, 1425, 1351, 1300, 1240, 848, 767 cm^{-1}; NMR δ 2.78 (s, 3 H), 7.50 (m, 3 H), 8.25 (m, 1 H).

Methyl 3-[1-(2-Pyridinyl 1-oxide)ethylidene]hydrazinecarbodithioate (II). A solution of 12.2 g (0.1 mol) of methyl hydrazinecarbodithioate[11] in 50 mL of MeOH was warmed to 50 °C with 13.7 g (0.1 mol) of 2-acetylpyridine 1-oxide. A vigorous exothermic reaction ensued, and the reaction mixture soon solidified. The solution was warmed on the steam bath for 0.5 h and then chilled, and the crystals that formed were collected and washed with cold MeOH, affording 18.8 g (78%) of pale yellow crystals of II, mp 160–162 °C. An analytical sample was prepared by recrystallization from MeCN: mp 161–162 °C; IR 3110, 3075, 2910, 1615, 1506, 1487, 1425, 1285, 1236, 851, 773, 755 cm^{-1}. Anal. ($C_9H_{11}N_3OS_2$) C, H, N, S.

3-Azabicyclo[3.2.2]nonane-3-thiocarboxylic Acid 2-[1-(2-Pyridinyl 1-oxide)ethylidene]hydrazide (14). The preparation of thiosemicarbazones derived from 2-acetylpyridine 1-oxide is exemplified by the following reaction. To a solution of 3.0 g (12.4 mmol) of II in 20 mL of MeOH was added 1.56 g (12.4 mmol) of 3-azabicyclo[3.2.2]nonane, and the solution was heated at reflux for 6 h. The crystals that separated from the cooled solution were collected and washed with MeOH, affording 3.59 g (91%) of stout orange needles of **14**. An analytical sample was preparaed by recrystallization from EtOH: IR 3085, 2930, 2860, 1575, 1535, 1432, 1225, 877, 751 cm^{-1}. Anal. ($C_{16}H_{22}N_4OS$) C, H, N, S.

Methyl 3-[1-(2-Pyridinyl 1-oxide)ethyl]hydrazinecarbodithioate (IV). A suspension of 24.1 g (0.1 mol) of II in 10 mL of EtOH was treated portionwise with 5.96 g (0.16 mol) of $NaBH_4$, and the mixture was stirred at room temperature for 1 h. To the resulting solution was added 250 mL of H_2O and 11 mL of glacial HOAc. An oil separated which crystallized upon cooling and scratching. The product was collected and then washed with H_2O, giving 22.0 g (92%) of colorless plates of IV, mp 182 °C dec. An analytical sample was prepared by recrystallization from EtOH: mp unchanged; IR 3250, 3130, 1494, 1482, 1427, 1334, 1227, 1218, 963, 848, 769 cm^{-1}. Anal. ($C_9H_{13}N_3OS_2$) C, H, N, S.

3-Azabicyclo[3.2.2]nonane-3-thiocarboxylic Acid 2-[1-(2-Pyridinyl 1-oxide)ethyl]hydrazide (19). To a solution of 400 mg (1.64 mmol) of IV in 8 mL of EtOH was added 206 mg (1.64 mmol) of 3-azabicyclo[3.2.2]nonane, and the resulting solution was heated at reflux for 4 h. The latter was chilled, and the colorless crystals that separated were collected and washed with cold MeOH, affording 460 mg (87%) of **19**, mp 188 °C dec. An analytical sample was prepared by crystallization from MeCN: mp 189 °C dec; IR 3172 (NH), 2935, 2865, 1487, 1436, 1348, 1226, 838, 777 cm^{-1}. Anal. ($C_{16}H_{24}N_4OS$) C, H, N, S.

Chloro[*N*,*N*-3-azabicyclo[3.2.2]nonane-3-thiocarbohydrazonato][1-(2-pyridinyl 1-oxide)ethylidene]copper(II) (15). A solution of 800 mg (2.5 mmol) of **14** in 125 mL of EtOH was combined with a solution of 426 mg (2.5 mmol) of $CuCl_2 \cdot 2H_2O$ in 10 mL of EtOH and heated to reflux, causing bronze-colored plates to separate within a short period of time. The mixture was allowed to cool to room temperature, and the product was collected, giving 910 mg (88%) of chloro[*N*,*N*-3-azabicyclo[3.2.2]-nonane-3-thiocarbohydrazonato][1-(2-pyridinyl 1-oxide)-ethylidene]copper(II): IR 3075, 2920, 2860, 1467, 1367, 1274, 1200, 1145, 825, 780 cm^{-1}. Anal. ($C_{16}H_{23}ClCuN_4S$) C, H, Cl, Cu, N, S.

Biological Methods. In vivo testing of the compounds described herein was conducted at the Leo Rane Laboratory, University of Miami, Miami, FL, against a drug-sensitive strain of *Plasmodium berghei* (strain KBG 173) in mice. Details of the test procedure were given by Osdene, Russell, and Rane.[22]

In vitro testing was conducted by the Department of Parasi-

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tology of this Division. Antimalarial activity against *Plasmodium falciparum* was determined by the semiautomated system described by Desjardins et al.[23] Two strains of the parasite were utilized, i.e., the multidrug-resistant Vietnam Smith strain[24] and the chloroquine-susceptible, pyrimethamine-resistant Camp strain.[25] Compounds were initially dissolved in a 50:50 (v/v) mixture of Me_2SO and EtOH to a concentration of 1 mg/mL. Subsequent dilutions were made with culture medium. [G-^{3}H]Hypoxanthine was diluted in culture medium to a concentration of 10 μCi/mL. Twenty-five microliters of this solution was added to each well of a 96-well microtiter plate. Incorporation of radioactivity by the parasites served as an index of parasite viability and antimalarial activity. Each drug was serially diluted 2-fold for a total of seven concentrations over a 64-fold range. Four wells of each microtiter plate contained chloroquine and mefloquine as controls. Plates were incubated at 37 °C for 24 h under an atmosphere of 5% O_2–5% CO_2–90% N_2. At this time, each well was treated with the [G-^{3}H]hypoxanthine, and incubation continued for an additional 18 h. The contents of each well were then collected on paper, and the paper was washed and subsequently dried at 80 °C for 1 h. Dried filter disks were individually counted in minivials containing a xylene-based scintillation fluid. Radioactivity was assayed in a Searle Delta 300 scintillation spectrometer to a counting error of 1%. The data were analyzed by a nonlinear regression analysis to obtain the 50% inhibitory dose (ID_{50}), the drug concentration corresponding to a 50% inhibition of the uptake of radiolabeled hypoxanthine by the parasites.

Registry No. 1, 87587-01-7; 2, 87587-02-8; 3, 87587-03-9; 4, 87587-04-0; 5, 87587-05-1; 6, 87587-06-2; 7, 87587-07-3; 8, 87587-08-4; 9, 87587-09-5; 10, 87587-10-8; 11, 87587-11-9; 12, 87587-12-0; 13, 87587-13-1; 14, 87587-14-2; 15, 87587-22-2; 16, 87587-15-3; 17, 87587-16-4; 18, 87587-17-5; 19, 87587-18-6; 20, 87587-19-7; 21, 87587-20-0; II, 87587-00-6; IV, 87587-21-1; dimethylamine, 124-40-3; pyrrolidine, 123-75-1; piperidine, 110-89-4; hexahydroazepine, 111-49-9; 2-methylpiperidine, 109-05-7; 3-methylpiperidine, 626-56-2; 4-methylpiperidine, 626-58-4; thiomorpholine, 123-90-0; 1-piperazinecarboxaldehyde, 7755-92-2; ethyl 1-piperazinecarboxylate, 120-43-4; 1-(2-pyridinyl)piperazine, 34803-66-2; 1-phenylpiperazine, 92-54-6; 2,6-dimethylmorpholine, 141-91-3; 3-azabicyclo[3.2.2]nonane, 283-24-9; methyl hydrazinecarbodithioate, 5397-03-5; 2-acetyl-1-pyridinone, 2457-50-3; 2-acetylpyridine, 112-62-9.

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Nucleosides. 129. Synthesis of Antiviral Nucleosides: 5-Alkenyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracils

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Synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracils containing a vinyl (**4a**), 2-halovinyl (**4b–d**), or ethyl substituent at C-5 was achieved. These nucleosides were found to be about a log order less active than 2′-fluoro-5-iodo-*ara*-C (FIAC) against HSV-1, but they are much less cytotoxic against normal human lymphocytes than FIAC. Nucleosides **4a** and **4e** showed good activity against HSV-1 (ED_{50} = 0.16 and 0.24 μM, respectively) and HSV-2 (ED_{50} = 0.69 and 0.65 μM) with very little cytotoxicity (ID_{50} > 100 μM).

The discovery[1,2] of the potent and selective anti herpes virus activity of 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (2′-fluoro-5-iodo-*ara*-C or FIAC)[1] and its clinical efficacy in phase 1 studies[3] of immunosuppressed patients with advanced cancer experiencing acute herpes virus infections led us to synthesize analogues of FIAC and test their antiviral activity. Among the analogues tested, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine (2′-fluoro-5-methyl-*ara*-U or FMAU)[4] was found to be about equal to FIAC in potency against herpes simplex virus type 1 (HSV-1) in vitro, yet it is far superior to it in vivo.[5,6] Not only is FMAU antiherpetic, but it is also antileukemic in mice at high dose levels.[7]

Our previous structure–activity relationship studies[1,4] showed the importance of substituents at C-5 and C-2′ of arabinosylpyrimidine nucleosides in determining anti herpes virus and cytotoxic activities. Thus, the fluoro substituent in the 2′-"up" arabino configuration, in general, brings about more potent and selective inhibitory activity against replication of herpes viruses than a hydrogen,[1] hydroxyl,[1] or other halogeno[4] substituent.

Recent reports on the potent antiherpetic activity of 5(*E*)-(halovinyl)-2′-deoxyuridines[8] and -*ara*-U[9] prompted us to prepare several 2-fluoroarabinosyl analogues containing 5(*E*)-(halovinyl)uracil for studies of their antiviral activity. These compounds were tested against both

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